AMERICAN ACADEMY™ OF OPHTHALMOLOGY

# Whole Genome Sequencing Increases Molecular Diagnostic Yield Compared with Current Diagnostic Testing for Inherited Retinal Disease

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***Purpose:*** To compare the efficacy of whole genome sequencing (WGS) with targeted next-generation sequencing (NGS) in the diagnosis of inherited retinal disease (IRD).

***Design:*** Case series.

***Participants:*** A total of 562 patients diagnosed with IRD.

***Methods:*** We performed a direct comparative analysis of current molecular diagnostics with WGS. We retrospectively reviewed the findings from a diagnostic NGS DNA test for 562 patients with IRD. A subset of 46 of 562 patients (encompassing potential clinical outcomes of diagnostic analysis) also underwent WGS, and we compared mutation detection rates and molecular diagnostic yields. In addition, we compared the sensitivity and specificity of the 2 techniques to identify known single nucleotide variants (SNVs) using 6 control samples with publically available genotype data.

***Main Outcome Measures:*** Diagnostic yield of genomic testing.

***Results:*** Across known disease-causing genes, targeted NGS and WGS achieved similar levels of sensitivity and specificity for SNV detection. However, WGS also identified 14 clinically relevant genetic variants through WGS that had not been identified by NGS diagnostic testing for the 46 individuals with IRD. These variants included large deletions and variants in noncoding regions of the genome. Identification of these variants confirmed a molecular diagnosis of IRD for 11 of the 33 individuals referred for WGS who had not obtained a molecular diagnosis through targeted NGS testing. Weighted estimates, accounting for population structure, suggest that WGS methods could result in an overall 29% (95% confidence interval, 15−45) uplift in diagnostic yield.

***Conclusions:*** We show that WGS methods can detect disease-causing genetic variants missed by current NGS diagnostic methodologies for IRD and thereby demonstrate the clinical utility and additional value of WGS. *Ophthalmology 2016;123:1143-1150 © 2016 by the American Academy of Ophthalmology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).*

*Supplemental material is available at www.aaojournal.org.*

Defining, with precision, the molecular genetic basis of ophthalmic disorders has a profound influence on clinicians' ability to diagnose, counsel, treat, and manage their patients.[1] Inherited retinal dystrophies (IRDs) are a diverse group of genetic disorders associated with visual impairment. They cause visual impairment in more than 2 million people worldwide and show extreme clinical and genetic heterogeneity.[2] As such, the clinical application of next-generation sequencing (NGS), a relatively recent technological advance that allows fast and cost-effective detection of genetic variation through parallel sequencing of multiple copies of fragmented DNA,[3] has transformed IRD discovery programs and genomic diagnostics, as well as for other Mendelian disorders.[4] Next-generation DNA sequencing techniques permit the analysis of genetic variants across multiple areas of the genome in a single procedure. To date, the application of NGS in the clinic largely has been limited to targeted techniques such as custom gene panels[5−7] and whole exome sequencing (WES),[8,9] which primarily restrict the analysis of genetic variation to protein-coding regions of the genome. However, these techniques are limited by the completeness of the genetic variation that can be detected, including reliable identification of large genomic deletions or duplications that encapsulate protein-coding regions and pathogenic variants in noncoding regions of genes. Such limitations are less evident from whole



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genome sequencing (WGS), an approach capable of detecting all types of variation across the complete human genome.[10] Targeted NGS techniques are the most commonly used genomic diagnostic test for IRD, but decreasing costs and increasing data interpretability have made WGS a realistic prospect for diagnostic use in the clinic.[11–14] This is exemplified by recent large-scale WGS cohorts, for example, the "100,000 Genomes Project" in England.[15] However, the comparative benefits of this technology, in respect to current diagnostic services, have yet to be truly delineated. The objective of this study is to identify the additional clinical advantages of WGS for individuals with IRD. We report the clinical findings from a retrospective review of 562 individuals referred for targeted NGS and a paired head-to-head comparison of targeted NGS and WGS for 46 unrelated individuals with clinical indications of IRD.

## Methods

### Sampling and Study Design

**Targeted Next-Generation Sequencing Testing.** The retrospective review included the first 562 individuals referred with IRD for clinically accredited targeted NGS. All individuals underwent clinical analysis of genetic variation within 105 genes known to underpin IRD (Table 1, available at www.aaojournal.org).[5] The included individuals were not knowingly related and had been referred from worldwide ophthalmic institutions. We analyzed the information available in the molecular diagnostic report and genetic variant files, including analysis of variant consequences and pathogenicity, clinical outcome, and carrier status. Phenotype–genotype correlations were elucidated from the scientific literature, including those referenced at the Retinal Information Network.[16] All analyses were conducted at the UK Manchester Centre for Genomic Medicine (MCGM).

**Comparison of Whole Genome Sequencing and Targeted Next-Generation Sequencing Testing: Variant Detection Accuracy.** To determine the ability of targeted NGS and WGS to detect single nucleotide variants (SNVs), we performed independent assessments of the *sensitivity* and *specificity* of the 2 techniques using control samples. We obtained control samples from the Coriell Institute for Medical Research Biorepositories in May 2013, which had been anonymized with unique catalog identifiers. Ethical permission was granted for the use of control samples to improve genomic diagnostic services, in line with the National Human Genome Research Institute Assurance Form for Biomaterials (B-031709, available at http://www.catalog.coriell.org). All control samples had publically available genotype data generated through the Illumina OMNI v2.5 microarray, a technique that identifies genotypes at approximately 2.5 million prespecified locations across the genome. We compared genotypes from the Illumina OMNI v2.5 microarray (available for each sample at ftp://ftp.sanger.ac.uk) with genotype calls from the targeted NGS and WGS pipelines. This was performed for 4 samples using targeted NGS through the Illumina HiSeq sequencing platform and 6 samples using WGS.

We calculated the *sensitivity* and *specificity* of targeted NGS and WGS to detect SNVs compared with the Illumina OMNI v2.5 microarray as described previously: *sensitivity*, the ability to detect SNVs identified by the Illumina OMNI v2.5 microarray, and *specificity*, the ability to identify homozygous reference sites identified by the Illumina OMNI v2.5 microarray.[17] For the clinically analyzed region of 105 genes surveyed by both targeted NGS and WGS, we were able to compare genotypes with the Illumina OMNI v2.5 microarray for 2714 genotyped sites using targeted NGS (616 SNVs and 2098 homozygous reference sites) and 4166 genotyped sites using WGS (928 SNVs and 3238 homozygous reference sites). All discordant sites were assessed via Sanger sequencing,[18] the "gold standard" genotyping technique.

**Comparison of Whole Genome Sequencing and Targeted Next-Generation Sequencing Testing: Diagnostic Utility.** The major motivation for this research was to assess whether WGS alters the number of individuals with a molecular diagnosis in comparison with currently delivered targeted genomic diagnostics. To address this question, we performed a paired head-to-head comparison between targeted NGS and WGS for 46 unrelated individuals with a clinical indication of IRD. All 46 individuals had been phenotypically assessed in a single institution. The cohort referred for WGS included the suite of possible clinical outcomes from targeted NGS testing (Fig 1). For each of the 46 individuals, we undertook consecutive genomic screening: first, targeted NGS, and second, targeted analysis of WGS data. For each individual, we assessed whether targeted NGS and WGS identified the same clinically relevant mutations and achieved the same clinical outcome.

Ethical permission for WGS was sought and granted for a single center, MCGM. Ethics Committee approval for this study was obtained through the REGARD study (Greater Manchester West Research Ethics Committee reference number: 11/NW/0421). Of the 562 individuals referred for targeted NGS testing, 126 were referred by clinicians at the MCGM, and 59 had appropriate consent for research genomics through the REGARD study. We excluded 13 of the 59 individuals on the basis of the quality and

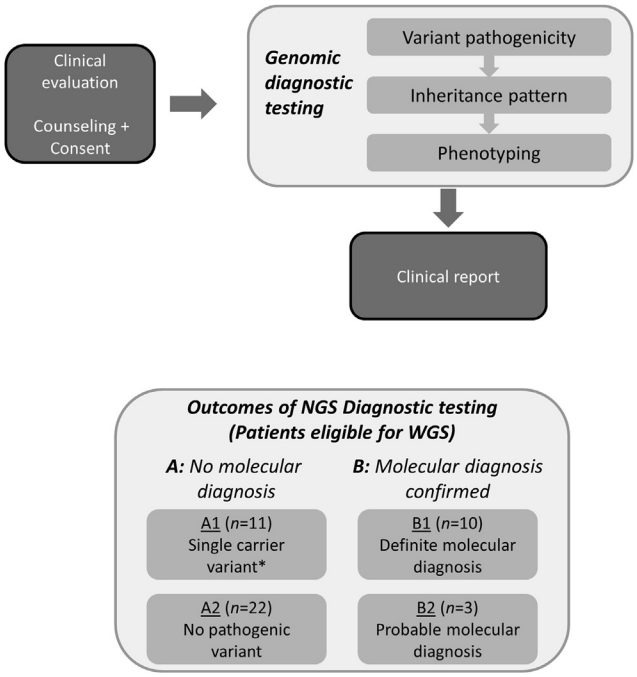


**Figure 1.** Data analysis and study design summary. Overview of targeted next-generation sequencing (NGS) diagnostic testing. *Single carrier variant defined as an individual with a pathogenic heterozygous variant found in a gene relevant to their clinical indication of inherited retinal disease (IRD) that is known to cause recessively inherited disease. WGS = whole genome sequencing.